

CUCURBITACIN-ADULTERATED DIET IS AVOIDED BY CAPTIVE EUROPEAN STARLINGS

J. RUSSELL MASON, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Denver Wildlife Research Center, % Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308
THOMAS TURPIN, Entomology Extension, Purdue University, West Lafayette, IN 47907

Abstract: Cucurbitacins are bitter-tasting glycosides present in many plants, especially those belonging to the family Cucurbitaceae. Because cucurbitacins show promise as a new category of agricultural insecticides, we designed an experiment to investigate whether birds would readily ingest treated food. Ground buffalo gourd (*Cucurbita foetidissima*) containing high (≥ 4 mg/g of gourd) levels of cucurbitacin was added to otherwise palatable feed and presented to European starlings (*Sturnus vulgaris*) in 2-cup and 1-cup tests. Reliable avoidance of treated feed was observed in both cases. It appears unlikely that buffalo gourd will be ingested accidentally by birds, presumably because of high concentrations of cucurbitacin. Instead, these materials might represent a new bird repellent and an agricultural insecticide with low hazard to nontarget bird species.

J. WILDL. MANAGE. 54(4):672-676

Cucurbitacins are triterpenoid glycosides that occur chiefly in plants belonging to the Cucurbitaceae and Cruciferae families (Guha and Sen 1975, Robinson 1983). To date, 17 cucurbitacins have been reported (Guha and Sen 1975), with the highest concentrations of the chemical occurring in roots and fruits (Rehm et al. 1957). These triterpenes are extremely bitter (David and Vallance 1955), and human taste detection thresholds are as low as 1 ppb (Metcalf et al. 1981).

The available evidence suggests that cucurbitacins protect plants against attack by herbivorous insects (Metcalf 1985). Experiments have shown that many such herbivores (e.g., the leaf beetles *Phyllotreta nemorum*, *P. undulata*, *P. tetrastigma*, *Phaedon cochleariae*, *P. crucifer-*

ae, and *Cerotoma trifurcata*) are repelled by the presence of cucurbitacins in the diet (Neilson et al. 1977, Metcalf et al. 1980). These substances, however, are not universally repellent. A variety of species, including economically important pests, such as the northern corn rootworm (*Diabrotica undecimpunctata*), are able to forage on Cucurbitaceae and respond to cucurbitacins in the diet as feeding stimulants (Metcalf 1985).

Cucurbitacins also may act as a defense against vertebrate herbivores (Metcalf 1985). However, the flavor, per se, of these compounds may not be strongly aversive. Severe accidental poisonings of livestock (Watt and Breyer-Brandwijk 1962) and humans (Ferguson et al. 1983) have been reported. Possibly, cucurbitacins-induced

gastrointestinal malaise serves as the basis for learned avoidance.

Because cucurbitacins have been considered for use as agricultural insecticides (Metcalf et al. 1983, Shaw et al. 1984), we designed an experiment to assess empirically the potential hazard of these substances to nontarget vertebrates. Ground buffalo gourd containing high levels of cucurbitacins was added to an otherwise palatable diet and then presented to European starlings in 1-cup and 2-cup tests.

MATERIALS AND METHODS

General.—Forty starlings were captured in mist-nets set up in the vicinity of Sandusky, Ohio and were transported to the Monell Chemical Senses Center, Philadelphia, Pennsylvania. Upon arrival, each bird was weighed, banded, and individually caged (61 × 36 × 41 cm) in a room with a 14:10 light:dark cycle and a constant ambient temperature of 23 C. Water was freely available, and before the experiments began, birds were permitted free access to granulated Purina Flight Bird Conditioner (Purina Mills Inc., St. Louis, Mo.) and crushed oyster shell grit, subsequently referred to as feed.

Stimuli.—Buffalo gourd is grown as a semi-domesticated crop in the American Southwest (Metcalf 1985), and it represents a convenient source of cucurbitacins (Berry et al. 1978). Gourds were dried and ground to a fine powder. The concentration of cucurbitacin in the powder was about 4.0 mg/g (Berry et al. 1978). Pure cucurbitacins were not used because there is no commercial source (Metcalf 1985). This powder (at 3 different concentrations, see below) was suspended in propylene glycol (Aldrich Chemical Co., St. Louis, Mo., CAS #57-55-6). The suspension was added to granulated feed at a rate of 1 mL of suspension per 100 g of feed. These procedures were used in an attempt to assure that the powder was evenly mixed with feed and that it adhered to the food particles. No attempt was made to assay cucurbitacin concentrations in prepared food samples.

Procedure.—After 2 weeks of adaptation to laboratory conditions, the 40 birds were randomly assigned to 2 cohorts ($n = 20/\text{cohort}$). On each of the 5 pretreatment days that followed, all food was removed from the cages within 1 hour of the onset of light, and the birds were given 2-hour access to 40 g of feed presented in 1 (cohort A) or 2 (cohort B) metal feeding cups (40 g or 20 g of feed/cup, respec-

tively) positioned in the front of the center of each cage. At the end of each testing session, consumption and spillage by each bird was recorded. Maintenance diet (feed) was then returned to the cages, and the birds were left undisturbed until the following day.

At the end of pretreatment period, a 5-day treatment period began. The 20 birds in each cohort were randomly assigned to 4 groups ($n = 5$ birds/group). For 1-cup tests, different groups were presented with feed containing 0.0, 1.25, 2.5, or 5.0% (g/g) buffalo gourd. For 2-cup tests, different groups were presented with 0.0, 1.25, 2.5, or 5.0% (g/g) gourd in feed in 1 cup and plain feed in another. The cup containing the gourd feed sample for each bird was randomly determined, and the left-right position of that sample was alternated daily. Treatment sessions were 2 hours long, and at the end of each session, consumption and spillage were recorded. During each session, the maintenance diet was removed from the cages; at the end of each session, this diet was returned. Birds were reweighed at the end of the experiment and subsequently were observed for 4 weeks before their release.

Analyses.—Pretreatment and treatment data in 2-cup tests were analyzed separately in 3-factor ANOVA's with repeated measures over days and cups. Subsequently, we calculated preference ratios for the treatment period by dividing consumption of treated feed by total consumption on each treatment day. These ratios were evaluated in a 2-factor ANOVA with repeated measures over days. In all cases, Tukey Honestly Significant Difference (HSD) post hoc tests (Winer 1962:198) were used to isolate significant differences among means subsequent to the omnibus procedures ($P < 0.05$).

For 1-cup tests, a 3-factor ANOVA with repeated measures over periods (pretreatment vs. treatment) and days was used to evaluate results. Tukey HSD tests were used to isolate significant differences among means ($P < 0.05$).

RESULTS

Two-Cup Tests.—There were no significant differences in the analysis of pretreatment data. Therefore, these results are not reported here. Analysis of treatment consumption, however, revealed significant differences in mean total consumption among days ($F = 3.2$; 4,64 df; $P < 0.02$) and between cups ($F = 82.9$; 1,16 df; $P < 0.00001$). Also, there was a significant in-

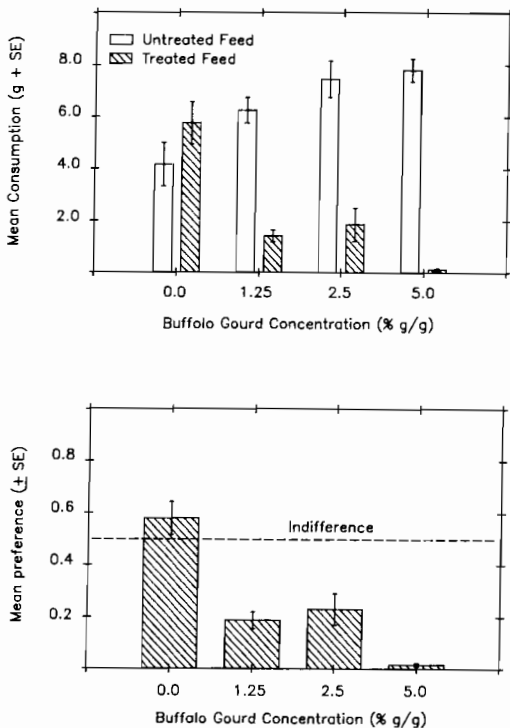


Fig. 1. Mean consumption (top panel) of gourd-treated and untreated feed in 2-cup tests. Each of the 4 groups was presented with a different gourd concentration. Mean preference ratios (bottom panel) were calculated for the treatment period on the basis of 2-cup tests results. A ratio of 1.0 reflects absolute preference for gourd-treated feed, a ratio of 0.0 absolute rejection of gourd-treated feed, and a ratio of 0.5 indifference. Capped vertical lines represent standard errors of the means.

teraction between concentrations and cups ($F = 19.5$; 3,16 df; $P < 0.0001$) (Fig. 1). There were no other significant effects ($P > 0.10$).

Regarding the days main effect, post hoc tests showed that mean consumption was significantly higher on Day 3 (5.3 ± 0.2 [$\bar{x} \pm \text{SE}$] g) than on Day 1 (3.8 ± 0.6) or Day 4 (3.5 ± 0.4). Intermediate levels of consumption were recorded on Day 2 (4.7 ± 0.6) and Day 5 (4.4 ± 0.6). Regarding the cups main effect, mean consumption of treated feed (2.5 ± 0.2 g) was significantly less than that of untreated feed (5.9 ± 0.2 g).

Examination of the concentration by cup interaction showed that all experimental groups (1.25, 2.5, 5.0% g/g) ate significantly less treated than untreated feed. Control group birds (0.0%) ate equivalent amounts from both cups.

Analysis of preference ratios revealed significant differences among concentrations ($F = 12.0$; 3,16 df; $P < 0.0004$) (Fig. 1). There were no

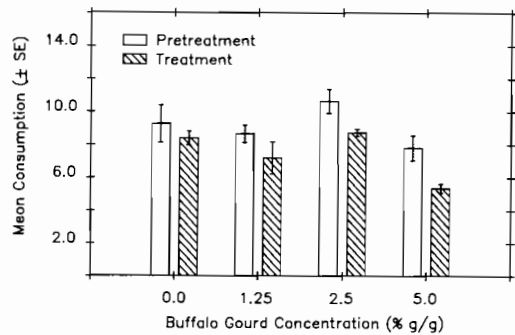


Fig. 2. Mean consumption of gourd-treated feed (hatched bars, treatment trials) and plain feed (open bars, pretreatment trials) in 1-cup tests. Capped vertical lines represent standard errors of the means.

other significant effects ($P > 0.10$). Post hoc examination showed that the mean preference ratio for the control group (0.525 ± 0.059) was significantly higher than mean preference ratios for any gourd-treated feed group (1.25%: 0.181 ± 0.03 ; 2.5%: 0.178 ± 0.034 ; 5.0%: 0.015 ± 0.022). In addition, the mean preference ratio for the group given 5.0% gourd was significantly lower than that for any other group.

One-Cup Tests.—There were significant differences among concentrations ($F = 4.5$; 3,16 df; $P < 0.018$), between periods ($F = 46.8$; 1,16 df; $P < 0.00001$), and among days ($F = 5.5$; 4,64 df; $P < 0.001$). Although the between period difference appeared greatest for birds in the 5.0% group (Fig. 2), no interaction terms were significant ($P > 0.20$).

Post hoc examination of the concentration main effect showed that birds presented with 5.0% gourd ate significantly less (6.6 ± 0.22 g) than birds presented with any other concentration (0.0%: 9.6 ± 0.34 g; 1.25%: 8.8 ± 0.30 g; 2.5%: 7.9 ± 0.18 g). In addition, birds presented with 2.5% gourd ate significantly less than the 0.0% control group. Regarding the main effect for periods, significantly less was consumed during the treatment period (7.4 ± 0.2) than during the pretreatment period (9.1 ± 0.16).

Evaluation of the days main effect indicated that consumption was significantly higher on Day 2 and Day 3 (8.7 ± 0.27 and 8.9 ± 0.35 , respectively), than on Day 1 (7.3 ± 0.31). Intermediate levels of consumption were recorded on Days 4 and 5 (8.1 ± 0.38 and 8.2 ± 0.24 , respectively).

Changes in mass of birds during the course of the experiment (76.4 ± 1.1 g vs. 77.6 ± 1.0 g) were not significant, and no mortalities were

recorded either during the experiment or during the 4-week observation period that followed.

DISCUSSION AND MANAGEMENT IMPLICATIONS

The results of both 2-cup and 1-cup tests show that buffalo gourd deters feeding by starlings. Further, 1-cup data suggest that ingestion of gourd, at least within the range of concentrations tested, presents little risk to birds. Although our data do not address the specific factors mediating repellency, we speculate that the astringent and/or bitter taste of cucurbitacins played a dominant role in avoidance. Given that about 4 mg of cucurbitacins are present in 1 g of buffalo gourd, our data suggest that starlings will avoid cucurbitacins at concentrations as low as 50 ppm in otherwise palatable feed.

Cucurbitacins have potential as new, biologically safe insecticides (Metcalf et al. 1983). These highly selective (Metcalf 1985) compounds can be used as lethal agents, as attractants for removal trapping or for disruption of mating, or as stimulants to enhance the ingestion of other insecticides. Our results suggest that cucurbitacins could also represent a safe alternative to some agricultural chemicals that present significant hazards to birds. At present, granular pesticide formulations and seed treatments are a major portion of the pesticide market as well as a principal source of income for major chemical companies (e.g., FMC 1988:5; Ciba-Geigy 1988: 8; DuPont 1989:4; American Cyanamid 1990: 29). A number of these materials are dangerous to some bird species (Best et al. 1990). Although particle size, texture, and the color of granular formulations may influence the hazard of agricultural chemicals to birds, the results of our 2-cup tests suggest that, when alternatives are available, even relatively low levels of cucurbitacins may inhibit ingestion of treated particles.

Several important issues remain to be addressed before field trials are attempted. First, our assumption that cucurbitacins, and not some other chemical, caused the birds to avoid food containing buffalo gourd needs to be tested. It also will be necessary to evaluate whether cucurbitacins are broadly repellent to many bird species because there might be species differences in avian responsiveness to bitter and/or astringent substances (Espaillat and Mason 1990). Starlings were used in our experiment because of their relatively acute taste capabilities, but

species such as red-winged blackbirds (*Agelaius phoeniceus*) are less sensitive to chemical stimuli (Espaillat and Mason 1990) and may be less responsive to cucurbitacins. Finally, the cucurbitacins concentrations we used were high relative to the quantities that effectively deter or enhance insect feeding (Metcalf 1985). Because our results indicate that even the lowest gourd concentration (1.25% g/g) was repellent to starlings and because 1 ppb cucurbitacins can be detected by humans, we speculate that concentrations below those we report could possess significant repellency.

LITERATURE CITED

- AMERICAN CYANAMID CO. 1990. Annual report. American Cyanamid Co. and Subsidiaries, Wayne, N.J. 57pp.
- BERRY, J. W., J. C. SCHEERENS, AND W. P. BEMIS. 1978. Buffalo gourd roots, chemical composition and seasonal changes in starch content. *J. Agric. Food Chem.* 26:345-356.
- BEST, L. B., R. C. WHITMORE, AND G. M. BOOTH. 1990. Use of cornfields by birds during the breeding season: the importance of edge habitat. *Am. Midl. Nat.* 123:84-99.
- CIBA-GEIGY. 1988. Annual report. Ciba-Geigy, Basel, Switzerland. 84pp.
- DAVID, A., AND D. K. VALLANCE. 1955. Bitter principles of cucurbitaceae. *J. Pharmacy Pharmacol.* 7:295-296.
- DUPONT DE NEMOURS AND CO. 1989. Annual report. DuPont De Nemours and Co., Wilmington, Del. 52pp.
- ESPAILLAT, J. E., AND J. R. MASON. 1990. Differences in taste preference between red-winged blackbirds (*Agelaius phoeniceus*) and European starlings (*Sturnus vulgaris*). *Wilson Bull.* 102: 292-299.
- FERGUSON, J. E., D. C. FISCHER, AND R. L. METCALF. 1983. A report of cucurbitacin poisoning in humans. *Cucurbit Genetics* 6:73-74.
- FMC CORP. 1988. Annual report. FMC Corp., Chicago, Ill. 44pp.
- GUHA, J., AND S. P. SEN. 1975. The cucurbitacins—a review. *Plant Biochem. J.* 2:12-28.
- METCALF, R. L. 1985. Plant kairomones and insect pest control. *Ill. Nat. Hist. Surv. Bull.* 33:175-198.
- , J. E. FERGUSON, D. FISCHER, R. LAMPMAN, AND J. ANDERSEN. 1983. Controlling cucumber beetles and corn rootworms with baits of bitter cucurbit fruits and root. *Cucurbit Genetics* 6:79-81.
- , E. R. METCALF, AND W. C. MITCHELL. 1981. Molecular parameters and olfaction in the oriental fruitfly *Dacus dorsalis*. *Proc. Natl. Acad. Sci. USA* 78:4007-4010.
- , R. A. METCALF, AND A. M. RHODES. 1980. Cucurbitacins as kairomones for diabroticite beetles. *Proc. Natl. Acad. Sci. USA* 77:3769-3772.
- NEILSON, J. K., L. M. LARSEN, AND H. J. SORENSON.

1977. Cucurbitacins E and I in *Iberis amara*, feeding inhibitors for *Phyllotreta nemorum*. *Phytochemistry* 16:1519-1522.
- REHM, S., P. R. EMLIN, A. D. J. NEEUSE, AND J. H. WESSELS. 1957. Bitter principles of the Cucurbitaceae VII. The distribution of bitter principles in the plant family. *J. Sci. Food Agric.* 8: 679-686.
- ROBINSON, T. 1983. The organic constituents of higher plants. Fifth ed. Cordus Press, Amherst, Mass. 353pp.
- SHAW, J. T., W. G. RUESINK, S. P. BRIGGS, AND W. H. LUCKMANN. 1984. Monitoring populations of corn rootworm beetles with a trap baited with cucurbitacin. *J. Econ. Entomol.* 77:1495-1499.
- WATT, J. M., AND M. G. BREYER-BRANDWIJK. 1962. The medicinal and poisonous plants of southern and eastern Africa. E. S. Livingston, Edinburgh, Scotland. 267pp.
- WINER, B. J. 1962. Statistical principles in experimental design. McGraw-Hill Book Co., New York, N.Y. 907pp.

Received 19 January 1990.

Accepted 14 May 1990.

Associate Editor: Conover.